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Reduced Antituberculosis Drug Concentrations in HIV-Infected Patients Who Are Men or Have Low Weight: Implications for International Dosing Guidelines

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Reduced antituberculosis drug concentrations may contribute to unfavorable treatment outcomes among HIV-infected patients with more advanced immune suppression, and few studies have evaluated pharmacokinetics of the first-line antituberculosis drugs in such patients given fixed-dose combination tablets according to international guidelines using weight bands. In this study, pharmacokinetics were evaluated in 60 patients on 4 occasions during the first month of antituberculosis therapy. Multilevel linear mixed-effects regression analysis was used to examine the effects of age, sex, weight, drug dose/kilogram, $CD4^+$ lymphocyte count, treatment schedule (5 versus 7 days/week), and concurrent antiretrovirals (efavirenz plus lamivudine plus zidovudine) on the area under the concentration-time curve from 0 to 12 h ($AUC_{0\text{-}12}$) of the respective antituberculosis drugs and to compare $AUC_{0\text{-}12}$s at day 8, day 15, and day 29 with the day 1 $AUC_{0\text{-}12}$. Median (range) age, weight, and $CD4^+$ lymphocyte count were 32 (18 to 47) years, 55.2 (34.4 to 98.7) kg, and 252 (12 to 500)/μl. For every 10-kg increase in body weight, the predicted day 29 $AUC_{0\text{-}12}$ increased by 14.1% (95% confidence interval [CI], 7.5, 20.8), 14.1% (95% CI, −0.7, 31.1), 6.1% (95% CI, 2.7, 9.6) and 6.0% (95% CI, 0.8, 11.3) for rifampin, isoniazid, pyrazinamide, and ethambutol, respectively. Males had day 29 $AUC_{0\text{-}12}$s 19.3% (95% CI, 3.6, 35.1) and 14.0% (95% CI, 5.6, 22.4) lower than females for rifampin and pyrazinamide, respectively. Level of immune suppression and concomitant antiretrovirals had little effect on the concentrations of the antituberculosis agents. As they had reduced drug concentrations, it is important to review treatment responses in patients in the lower weight bands and males to inform future treatment guidelines, and revision of doses in these patients should be considered.

It is critical that dosing of antituberculosis drugs is optimized since reduced drug concentrations may result in prolonged infectiousness, the emergence of drug-resistant organisms, treatment failure, or relapse. Fixed dose combinations (FDCs) are promoted for the treatment of drug-sensitive tuberculosis to prevent the emergence of drug resistance, to reduce pill burden, and to simplify operational aspects of drug supply and delivery to patients. The World Health Organization (WHO) recommends that adults be treated with 2 to 5 tablets per day depending on the weight of the patient (39). However, the drug concentrations achieved in patients dosed accordingly have not been evaluated across weight bands. Reduced drug exposure among HIV-infected patients (13, 19) may contribute to the worse treatment outcomes reported among patients with HIV-associated tuberculosis than for HIV-uninfected patients, notably when less robust antituberculosis regimens are used, treatment duration is 6 months or less, or intermittent dosing is employed (14, 16, 23, 31). There is concern that the greater risk of tuberculosis recurrence among HIV-infected patients with more advanced immune suppression (4, 24, 37) may, in part, be due to lower drug concentrations (26, 28, 33).

We sought to identify factors associated with pharmacokinetic variability of rifampin, isoniazid, pyrazinamide, and ethambutol in HIV-infected patients dosed with FDCs according to international recommendations. Because the pharmacokinetic study was nested within a randomized controlled trial evaluating early versus deferred antiretroviral therapy (ART), we were able to assess the effect of ART on the pharmacokinetics of the antituberculosis drugs.

MATERIALS AND METHODS

Patients and study treatments. Patients aged 18 to 65 years with HIV-1 infection and not previously treated for HIV were recruited between March 2007 and April 2008 at primary health care facilities in and around Durban, South Africa. All patients provided their written informed consent to participate. Patients with a history of drug-resistant tuberculosis or tuberculosis treatment in the preceding year, severe illness, an indication for medication that might interact with study medication, treatment-dependent diabetes mellitus, epilepsy, or a history of excessive alcohol consumption or drug abuse and pregnant women and women of childbearing age refusing to use contraceptive measures for the duration of the study were not enrolled. Patients weighing <30 kg, with hepatic transaminases >2.5 times the upper limit of normal (ULN), serum creatinine >1.5 times the ULN, neutrophils ≤1,200/μl, or hemoglobin <8 g/dl were excluded.

Sixty-two HIV-infected patients with smear-positive pulmonary tuberculosis were enrolled. Twenty patients with $CD4^+$ lymphocyte counts of <200/μl were started on ART (efavirenz at 600 mg daily, lamivudine at 150 mg twice daily, and zidovudine at 300 mg twice daily) after 14 days of antituberculosis therapy (ATT). Forty-two patients with 220 to 500 $CD4^+$

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TABLE 1 Baseline characteristics[a]

Characteristic	Early ART $n = 20$	$n = 20$	Delayed ART, $n = 20$
CD4 count	<200	220–500	220–500
Female, *n* (%)	13 (65%)	12 (60%)	7 (35%)
Age (yr)[b]	37 (32, 42)	30 (26, 35)	31 (25, 35)
Wt (kg)	55.1(47.0, 62.8)	56.4, (50.9, 62.9)	55.1 (51.9, 62.2)
BMI (kg/m^2)	21.18 (19.23, 25.27)	22.76 (19.01, 24.76)	21.84 (19.20, 23.01)
ATT 5 days/week[b]	20 (100%)	15 (75%)	15 (75%)
Viral load ($\times 10^3$ copies/ml)[b]	210.0 (73.0, 625.0)	61.0 (22.7, 207.0)	38.7 (7.8, 196.4)
CD4$^+$ cells/μl[b]	110 (81, 157)	310 (272, 328)	320 (240, 348)
Hemoglobin (g/dl)	10.3 (8.8, 11.0)	10.1 (9.4, 12.4)	11.2 (9.7, 12.2)
White cells ($\times 10^9$/liter)	5.63 (3.95, 8.00)	6.30 (5.64, 8.85)	6.51 (5.81, 8.38)
Platelets ($\times 10^9$/liter)	345 (228, 428)	345 (283, 477)	356 (275, 494)
ALT (units/liter)[c]	16 (13, 26)	15 (13, 22)	17 (13, 28)
Creatinine (μmol/liter)	70 (67, 81)	73 (62, 91)	78 (72, 89)

[a] Characteristics are expressed as median IQR (unless otherwise indicated) of study participants by CD4$^+$ lymphocyte count stratum and treatment group (early versus delayed ART).
[b] Kruskal-Wallis $P < 0.05$.
[c] Normal range, 10 to 45 U/liter.

lymphocytes/μl were randomized to early introduction of ART (after 14 days of ATT) or deferred ART (ART was started after completion of ATT).

ATT was delivered in a 4-drug FDC supplied by the National Tuberculosis Control Programme (Rifafour e-275 [Sanofi-Aventis, South Africa] in 53 patients and Antib-4 [Rusan Pharma, India] in the remaining 7 patients; 2 patients were withdrawn [see below]). Each tablet contained rifampin at 150 mg, isoniazid at 75 mg, pyrazinamide at 400 mg, and ethambutol at 275 mg. Weight band-based dosing was used in line with WHO guidelines (39). Patients weighing 30 to 37 kg, 38 to 54 kg, 55 to 70 kg, and >70 kg were given doses of 2, 3, 4, and 5 tablets, respectively. ATT was administered 5 days/week, with a "drug holiday" on weekends in the majority of patients. Due to a change in dosing policy during the study period, 10 (17%) patients were treated with doses 7 days/week. For one patient the number of ATT doses per week was not recorded, and we inferred that 5 doses/week were taken based on the time that the patient entered the study. Treatment doses were observed by the study nurse or a treatment supporter.

Participants were admitted for pharmacokinetic evaluation on the 1st, 8th, 15th, and 29th days of treatment. ATT doses were taken after an overnight fast and observed by a study team member. Blood specimens collected just prior to the ATT doses and at 1, 2, 3, 4, 6, 8, and 12 h after dosing were immediately placed on ice and plasma separated by centrifugation within 30 min before storage at −70°C until analysis. The storage tubes containing the plasma samples were transferred to the analytical laboratory (Division of Clinical Pharmacology, University of Cape Town) in dry ice.

Pharmacokinetics. Rifampin, isoniazid, and pyrazinamide in plasma were assayed as previously described (18). Ethambutol was assayed by liquid chromatography (LC)-tandem mass spectrometry on an API 4000 LC mass spectrometer using a modification of the method of Conte et al. (8). The mobile phase consisted of gradient of 4 mM ammonium acetate in 0.05% trifluoroacetic acid and acetonitrile. Chromatography was performed on a Discovery HS F5 high-performance liquid chromatography (HPLC) column maintained at 25°C. Neostigmine served as the internal standard. Acetonitrile with 0.04% formic acid containing the internal standard was used to precipitate 50 μl of each sample, which was then centrifuged; 5 μl of the supernatant was injected onto the column. The lower limits of quantification (LLQ) were 0.2 mg/liter for pyrazinamide and 0.1 mg/liter for isoniazid, ethambutol, and rifampin. Inter- and intraday coefficients of variation were below 9% for all quality control samples. Drug concentrations >20% below the LLQ were assigned a value of 50% of the accepted lower limit if they occurred before the peak concentration (C_{max}). After C_{max}, the first concentration >20% below the LLQ was assigned a value of 50% of the accepted lower limit and subsequent samples were treated as missing data.

Statistical methods. A noncompartmental approach was used to calculate the pharmacokinetic measures. C_{max} was determined directly from the concentration-time data; area under the curve until the 12-h time point (AUC_{0-12}) was calculated using the linear trapezoidal rule. Half-life was defined as $\ln(2)/k_e$, where k_e equals the slope of the log-linear regression of the final 3 data points. Due to missing 12-h samples, in 3 instances the 12-h concentrations were imputed with an exponential extrapolation based on the prior 3 samples. Multilevel linear mixed-effects (MLME) regression was used to examine the effects of age, sex, baseline weight, drug dose per kilogram of body weight at baseline, baseline level of immune suppression (CD4$^+$ lymphocyte count), ATT dosing schedule (5 versus 7 days/week), concurrent first dose of antiretrovirals (day 15), and the presence of steady-state antiretroviral exposure (day 29) on the AUC_{0-12} of the respective antituberculosis drugs measured on days 1, 8, 15, and 29. The MLME model included a random effect for patient to account for the correlation in a patient's AUC_{0-12} over time. The effect of time was included as a categorical variable to allow AUC_{0-12} to vary in a nonlinear manner over time; day 8, 15, and 29 AUC_{0-12}s were compared to baseline AUC_{0-12}s (day 1). Model checking was based on the distribution of standardized residuals. The delta method was employed using R, version 2.10.1 (http://CRAN.R-project.org), to obtain approximate 95% confidence intervals (CIs) for the percent changes (25). As models using the untransformed data were unsatisfactory for isoniazid AUC_{0-12}, the natural logarithms of AUC_{0-12} were used and covariate effects were described as percent changes on the original scale. The Kruskal-Wallis rank test (continuous variables) and Pearson's chi-squared test (categorical data) were used to assess the equality of the population baseline variables across independent subgroups. Stata, version 11.0 (StataCorp., College Station, TX), was used to compute pharmacokinetic measures, summary statistics, statistical tests, and regression analyses.

RESULTS

Patients and treatments. Two patients with CD4$^+$ lymphocyte counts of 220 to 500/μl who withdrew before completion of pharmacokinetic evaluation on day 1 were excluded from the analysis. Baseline characteristics of the remaining 60 patients are shown in Table 1. Patients with more advanced HIV were older on average than patients with CD4$^+$ lymphocyte counts of >220/μl. There

TABLE 2 Median (IQR) C_{max}s and AUC_{0-12}s after 1 month of antituberculosis therapy by ART treatment group

Parameter	No. of patients	Rifampin	Isoniazid	Pyrazinamide	Ethambutol
C_{max} (mg/liter)					
$CD4^+$ count, 220–500, early ART	13	7.8 (6.6, 8.7)	1.5 (0.9, 2.1)	37.0 (31.4, 43.0)	3.0 (2.5, 3.6)
$CD4^+$ count, <220, early ART	19	7.0 (5.9, 9.5)	1.7 (1.2, 1.8)	34.7 (28.1, 40.4)	3.7 (3.1, 3.9)
$CD4^+$ count, 220–500, ART deferred	19	6.6 (5.6, 8.4)	2.0 (1.6, 2.5)	33.4 (26.2, 36.1)	2.6 (2.2, 3.0)
All groups combined	51	7.2 (5.9, 8.8)	1.7 (1.2, 2.2)	34.7 (28.1, 41.5)	3.0 (2.3, 3.8)
AUC_{0-12} (mg · h/liter)					
$CD4^+$ count, 220–500, early ART	13	39.6 (34.8, 56.7)	5.7 (2.5, 6.9)	293.0 (234.7, 337.7)	15.8 (14.1, 19.0)
$CD4^+$ count, <220, early ART	19	36.3 (30.3, 56.7)	4.4 (3.8, 8.6)	260.3 (228.0, 362.6)	19.9 (17.4, 21.7)
$CD4^+$ count, 220–500, ART deferred	19	31.4 (27.5, 43.8)	7.2 (5.1, 10.0)	228.4 (197.6, 278.8)	13.5 (12.2, 19.1)
All groups combined	51	36.3 (27.9, 48.0)	5.9 (3.8, 8.6)	260.3 (212.5, 337.2)	16.4 (12.9, 20.5)
Spearman correlation[a]					
All groups combined	51	0.83	0.80	0.91	0.83

[a] All correlations between C_{max} and AUC_{0-12} were significant at the 0.01 level.

were no significant differences in the baseline characteristics between patients randomized to early or deferred ART.

Among patients randomized to early ART ($CD4^+$ lymphocyte counts of 220 to 500/μl), four withdrew their consent to participate prior to completion of pharmacokinetic sampling (resulting in missing day 15 pharmacokinetic data for 3 patients and missing day 29 pharmacokinetic data for 4), two were withdrawn before day 8 due to serious adverse events (anemia and elevated amylase), and another patient was withdrawn after day 8 when the $CD4^+$ lymphocyte count was confirmed to be >500/μl. Among patients randomized to deferred ART, one patient was withdrawn after day 8, as the $CD4^+$ lymphocyte count dropped below 200/μl and ART was commenced.

Pharmacokinetics. More than 98% of the pharmacokinetic data for each drug was included in the analysis. Data were excluded due to errors in the time of dosing, missing samples, sample labeling errors, or undetectable drug during the entire sampling period. Excluding predose samples, 10.5, 25.2, 0.3, and 0.6% of the samples had concentrations of rifampin, isoniazid, pyrazinamide, and ethambutol, respectively, below the LLQ. For rifampin, 9% of 12-h samples were below the LLQ for patients weighing less than 55 kg (on 2 or 3 FDC tablets), and 3% of 12-h samples from patients weighing 55 kg or more (on 4 or 5 FDC tablets) were below the LLQ. The remaining concentrations below the LLQ were from the absorption phase. For isoniazid, 10, 16, 22, and 20% of 4-, 6-, 8-, and 12-h samples were below the LLQ, with a larger proportion of patients under 55 kg having 12-h samples below the LLQ.

C_{max} and AUC_{0-12} for the antituberculosis drugs after a month of treatment are presented in Table 2. The results of the MLME models used to estimate changes in AUC_{0-12} during the first month of treatment and the effects of patient and treatment factors on AUC_{0-12} are presented in Table 3. The fit of the models was assessed and found to be adequate. In spite of dosing according to weight band and adjustment for the effect of dose per kilogram of body weight by inclusion of the covariate dose/kg in the models, a reduction in weight was associated with reduced drug concentrations. Each 10-kg change in weight was positively associated with

TABLE 3 Estimated effects of time on antituberculosis treatment, concomitant ART, age, sex, baseline weight, baseline level of immune suppression ($CD4^+$ cell count), dosing schedule, and dose/kilogram of body weight on systemic exposure (AUC_{0-12}) to the antituberculosis drugs

	Estimated change in AUC_{0-12} (95% CI)			
Time of measurement	Rifampin	Isoniazid[a]	Pyrazinamide	Ethambutol
Day 8[b]	−14.98 (−18.87, −11.08)	8 (−3, 19)	44.47 (29.02, 59.91)	3.92 (2.79, 5.05)
Day 15[b]	−19.42 (−25.38, −13.45)	8 (−8, 27)	0.56 (−22.95, 24.06)	3.23 (1.48, 4.97)
Day 29[b]	−20.81 (−26.77, −14.85)	0 (−15, 18)	−1.45 (−24.95, 22.06)	2.81 (1.07, 4.55)
1st-dose ART[c]	0.48 (−6.49, 7.45)	−9 (−25, 10)	28.05 (0.68, 55.41)	1.16 (−0.88, 3.21)
Steady-state ART[d]	−4.48 (−11.53, 2.56)	−6 (−23, 13)	12.96 (−14.70, 40.61)	2.33 (0.26, 4.39)
Age (yr)	0.07 (−0.39, 0.52)	2 (−1, 4)	−1.44 (−3.06, 0.18)	0.02 (−0.13, 0.17)
Male sex	−8.61 (−14.98, −2.24)	−9 (−35, 28)	−40.52 (−63.19, −17.84)	−0.93 (−3.00, 1.13)
Wt/10 (kg)[e]	5.74 (3.13, 8.34)	14 (−1, 31)	16.63 (7.37, 25.89)	0.98 (0.13, 1.83)
$CD4^+$ count/50 (cells/μl)	−0.96 (−2.38, 0.47)	8 (1, 17)	−2.54 (−7.62, 2.55)	0.36 (−0.10, 0.82)
7 doses/week[f]	−6.55 (−14.50, 1.41)	15 (−25, 76)	33.84 (5.56, 62.12)	4.53 (1.95, 7.11)
Dose/wt (mg/kg)	2.94 (−0.50, 6.39)	40 (−3, 102)	8.65 (4.06, 13.23)	0.12 (−0.49, 0.73)

[a] AUC_{0-12} for isoniazid was transformed using the natural logarithm. Changes are given as percentages.
[b] Change from day 1.
[c] Independent effect of ART on day 15 versus all other AUC observations.
[d] Independent effect of ART on day 29 versus all other AUC observations.
[e] Change in AUC related to a 10-kg weight change.
[f] Change relative to 5 doses/week.

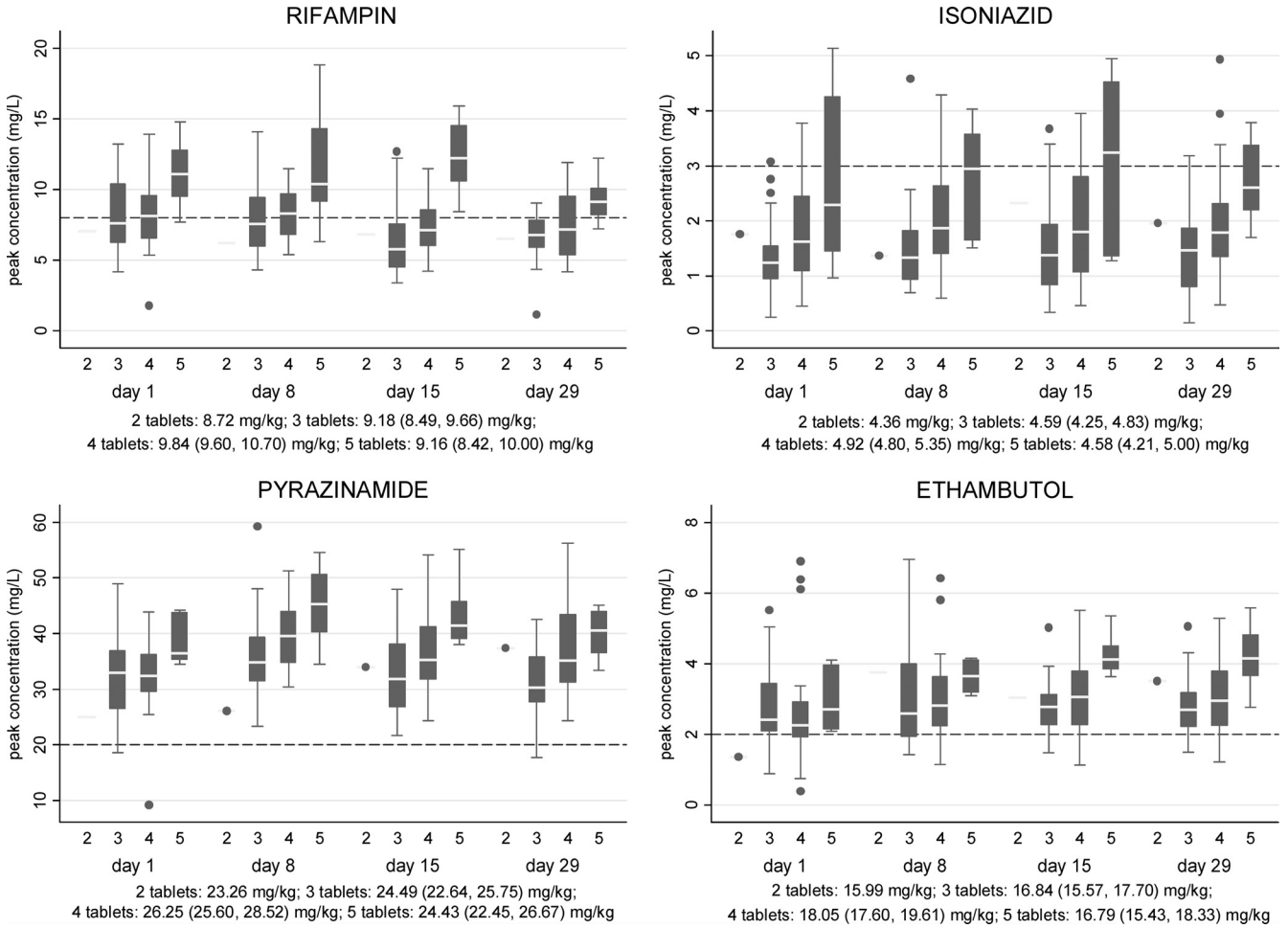

FIG 1 Peak concentrations (boxes show IQR divided by the median) for each of the antituberculosis drugs according to the different dosing weight bands (indicated by the number of tablets: 2, 3, 4, or 5) at each sampling day (days 1, 8, 15, and 29). Antituberculosis treatment was delivered to patients in 2 ($n = 1$ [2%]), 3 ($n = 26$ [43%]), 4 ($n = 26$ [43%]), or 5 ($n = 7$ [12%]) tablets according to baseline body weight. The median (IQR) doses for rifampin, isoniazid, pyrazinamide, and ethambutol in the respective weight bands are shown beneath each graph. The dashed lines indicate the lower limit of the target peak concentration ranges (27).

a change in the predicted day 29 $AUC_{0\text{-}12}$ of 14.1% (95% CI, 7.5, 20.8), 14.1% (95% CI, −0.7, 31.1), 6.1% (95% CI, 2.7, 9.6), and 6.0% (95% CI, 0.8, 11.3), respectively, for rifampin, isoniazid, pyrazinamide, and ethambutol. C_{max}s by weight band at each sampling occasion are shown in Fig. 1. Males had predicted reductions in $AUC_{0\text{-}12}$ of 19.3% (95% CI, 3.6, 35.1) for rifampin and 14.0% (95% CI, 5.6, 22.4) for pyrazinamide.

The level of immune suppression had no effect on rifampin, pyrazinamide, or ethambutol exposure. Isoniazid $AUC_{0\text{-}12}$ was reduced by an estimated 8.5% (95% CI, 0.6, 16.9) for each 50-$CD4^+$ lymphocyte/μl decline, after adjustment for other factors in the model. ART had limited effects: pyrazinamide $AUC_{0\text{-}12}$ transiently increased with the initial dose of ART, but this effect waned once patients were established on ART, and after 2 weeks of ART, ethambutol $AUC_{0\text{-}12}$ values were modestly increased. Variations in $AUC_{0\text{-}12}$ were observed over time for all the drugs except isoniazid. Rifampin $AUC_{0\text{-}12}$ declined by an estimated 14.98 (95% CI, 11.08, 18.87) mg · h/liter after 1 week of ATT. Further declines resulted in a reduction of 20.81 (95% CI, 14.85, 26.77) mg · h/liter by day 29. Pyrazinamide $AUC_{0\text{-}12}$ transiently increased after commencing ATT but waned by the 15th day of treatment to values similar to those after the first dose. Ethambutol concentrations increased during the first week of therapy and remained higher than day 1 concentrations at steady state. Patients who received 7 daily doses of ATT/week tended to have lower rifampin concentrations than patients given 5 doses a week with weekend treatment holidays. Conversely, pyrazinamide and ethambutol concentrations after 4 weeks of therapy were modestly higher among those who received 7 doses a week. Age was not associated with changes in antituberculosis drug concentrations.

DISCUSSION

Among our cohort of HIV-infected patients with tuberculosis who were dosed according to WHO-recommended weight bands (39), low-weight and male patients had reduced antituberculosis drug concentrations. Predictions based on our MLME models estimated rifampin $AUC_{0\text{–}12}$ to be approximately 2.4-fold higher in a female weighing 75 kg than in a male weighing 35 kg. For isoniazid and pyrazinamide, there was a 1.5-fold increase, and for ethambutol, there was a 1.4-fold increase. Weight was strongly

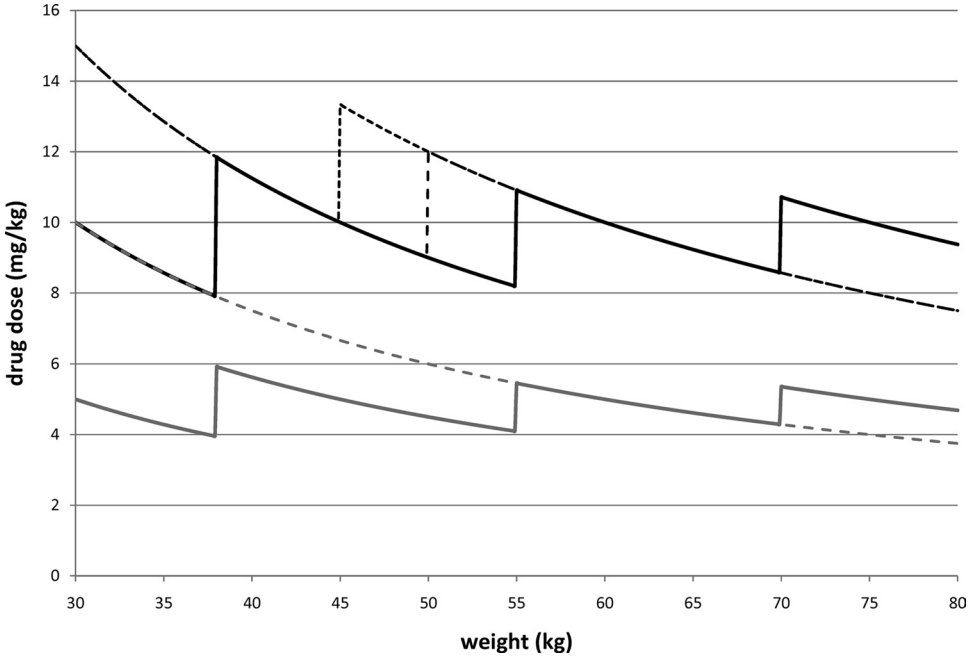

FIG 2 Doses of the key drugs rifampin and isoniazid, expressed in milligrams per kilogram of body weight as recommended by WHO guidelines (39) (rifampin, upper solid black line; isoniazid, lower solid gray line) and the American Thoracic Society (3) (rifampin, fine dotted line; isoniazid, gray dashed line) and those used in seminal early studies (10) (rifampin, black dashed line; isoniazid, gray dashed line).

associated with drug concentrations, most notably for the key drug rifampin. The effect was statistically significant for all the drugs except isoniazid, which showed a nonsignificant trend in the same direction ($P = 0.062$). The nonlinear relationship between drug clearance and weight partly explains this effect (2). Moreover, for many drugs eliminated predominantly by the liver, systemic clearance is correlated with lean body weight (21). In our cohort, patients receiving 2, 3, 4, and 5 FDC tablets had average body mass indexes (BMIs) of 16.1, 19.5, 22.9, and 30.8 kg/m^2, respectively. BMI could be substituted for weight in the MLME models describing AUC_{0-12} without substantially changing the estimates (data not shown). A previous report described a 29% reduction in the AUC of rifampin among subjects with a mean BMI of 14.8 kg/m^2 in comparison to subjects with BMIs of >18 kg/m^2 (30). Hence, wasted patients, who are more likely to have severe disease at presentation, are at greater risk of low antituberculosis drug concentrations. A disproportionate increase in rifampin concentrations at higher doses was described previously (1, 32). A population pharmacokinetic model in which increased doses of rifampin were associated with a shortened transit time prior to absorption suggests that saturation of the efflux transporter P-glycoprotein contributes to increased rifampin concentrations among patients given more tablets (6). Many studies underpinning current treatment guidelines employed higher doses of rifampin and isoniazid than those currently recommended for low-weight patients (Fig. 2). Few studies have evaluated outcomes in patients treated with the weight band-based doses currently recommended by the WHO. Our results suggest that weight, BMI, and sex should be evaluated as risk factors for poor treatment outcomes in such studies.

In keeping with previous reports, males had reduced rifampin and pyrazinamide concentrations compared to females (19, 36). Higher lean-body/total-weight ratios in males might partly account for this finding. Male sex has been associated with poor treatment outcomes (15, 20, 22). Our findings suggest that pharmacokinetics might play a role.

Reductions in rifampin bioavailability of 32 to 50% have been ascribed to HIV infection (5, 13, 19, 33). Pyrazinamide (33) and ethambutol (13, 19) concentrations may also be reduced among subjects with HIV, and similar trends have been described for isoniazid in association with diarrhea (13, 33). There is concern that patients with advanced HIV infection may have more extensive malabsorption due to HIV-mediated enteropathy and increased susceptibility to enteric infections. Our finding that the level of immune suppression has very little impact on the concentrations of the first-line antituberculosis drugs is reassuring. However, patients with advanced immune suppression were not well represented in our cohort; only 3 patients had <50 $CD4^+$ lymphocytes/μl. The presence of mild to moderate diarrhea may account for the modest reduction in isoniazid exposure among patients with lower $CD4^+$ cell counts, as 6 of 7 tuberculosis patients with diarrhea had $CD4^+$ lymphocyte counts of <200/μl.

The concomitant use of ART was not associated with reduced antituberculosis drug concentrations. Pyrazinamide AUC_{0-12} was transiently increased on initiation of ART, and ethambutol was modestly increased after 2 weeks of combined therapy. Although dose-related toxicity is a concern with both pyrazinamide and ethambutol, the increases are unlikely to be clinically important at the currently utilized doses.

Rifampin concentrations compared favorably to those reported for other African patients (5, 7, 19) and to those in HIV-infected patients (13, 29). Although 31 (61%) patients had day 29 rifampin C_{max}s below 8 mg/liter (the lower limit of the reference range [LLRR] [27]), only one patient had a C_{max} below 4 mg/liter.

Conversely, we found surprisingly low isoniazid concentrations in comparison to other studies (5, 13, 19). Forty-five (88%) patients had day 29 C_{max}s below the LLRR (3 mg/liter). As isoniazid degrades rapidly at temperatures of −20°C and above, we conducted a thorough audit of sample handling, storage, and shipping procedures. Furthermore, an independent laboratory repeated the isoniazid measurements in 2.5% of the samples and found concordant results. NAT2 gene polymorphisms vary widely between regions (38). Rapid acetylator genotypes are reported to occur in about 60% of South Africans (17, 34), but NAT2 genotype frequencies have not been reported among patients of Zulu ethnicity, which the majority of participants in our study represent. Interestingly, Kenyans were found to have similarly low isoniazid concentrations (7). One factor contributing to reduced isoniazid concentrations in our study is dose; 27 (45%) patients had isoniazid doses of <300 mg (in accordance with the WHO guidelines for patients weighing <55 kg). In most earlier studies, patients were given 300-mg doses daily regardless of weight. The half-life of isoniazid was within the expected range (day 29 median, 2.32 h; interquartile range [IQR], 1.40, 3.02). Although the MIC of *Mycobacterium tuberculosis* is typically expected to be low compared to peak isoniazid concentrations after a 300-mg dose, there is evidence to suggest that reduced isoniazid exposure is important for outcome: the therapeutic efficacy of a regimen including thiacetazone and isoniazid was reduced when the dosage of isoniazid was reduced from 300 mg to 200 mg a day (11), dosages of isoniazid of >150 mg daily are necessary for maximal early bactericidal activity (9), and maximal bactericidal effect in an *in vitro* model was associated with isoniazid exposures mimicking dosages of ≥150 mg/day (12).

The day 29 pyrazinamide C_{max} was >20 mg/liter (LLRR) in 50 (98%) patients but <35 mg/liter in 26 (51%) of our patients. Should the recently reported association between a C_{max} of <35 mg/liter and poor treatment outcome (5) be confirmed, higher doses of pyrazinamide should be considered, especially among patients in lower weight bands. Ethambutol concentrations compared favorably with reports of other patient cohorts (5, 19, 29, 40). Only 6 (12%) patients had C_{max}s of <2 mg/liter (LLRR) on day 29. Pyrazinamide and ethambutol displayed accumulation during the first week. That patients administered ATT 7 days/week had higher pyrazinamide and ethambutol AUC_{0-12}s than patients with a drug holiday on weekends is consistent with this finding. Interestingly, pyrazinamide concentrations declined after a week to baseline concentrations, suggesting induction of clearance processes. Although it is scientifically plausible that patients dosed 5 days/week tend to have higher rifampin concentrations, due to attenuation of autoinduction at weekends, the hypothesis needs to be evaluated in larger studies. Interestingly, rifampin concentrations declined significantly after the 8th day of treatment, suggesting that the autoinduction process may be incomplete after a week. This is supported by recent findings that the clearance of rifampin takes approximately 40 days to reach a steady state (35).

This study was not designed to investigate drug exposure-effect relationships but sought to identify factors associated with pharmacokinetic variability in tuberculosis patients with HIV at different levels of immune suppression. The importance of pharmacokinetic variability of the first-line antituberculosis drugs given in daily doses is inadequately evaluated. It is challenging to ascertain the contributions of individual drug components within ATT. Correlation between drug concentrations within the regimens complicates interpretation. A recent study associated pyrazinamide peak concentrations of <35 mg/liter with an increased risk of treatment failure or death (5). However, we found that the pyrazinamide AUC_{0-12} after 1 month of therapy was strongly correlated with rifampin and ethambutol (Spearman's rho = 0.623 and $P < 0.001$ and Spearman's rho = 0.542 and $P < 0.001$, respectively). Pyrazinamide and isoniazid AUC_{0-12}s were weakly correlated (Spearman's rho = 0.225 and $P = 0.113$). Rifampin AUC_{0-12} was weakly associated with isoniazid and ethambutol AUC_{0-12}s (Spearman's rho = 0.270 and $P = 0.055$ and Spearman's rho = 0.246 and $P = 0.082$, respectively), and isoniazid AUC_{0-12} was not associated with ethambutol AUC_{0-12} (Spearman's rho = 0.101 and $P = 0.483$).

Dosing guidelines for global use should include consideration of drug concentrations in other patient populations. A combined model-based pharmacokinetic meta-analysis using a population approach has the potential to provide insights into sources of variability across populations and hence to inform optimal dosing. Moreover, such megamodels could be extended to evaluate the relationships between pharmacokinetics, treatment outcomes, and risk factors such as weight, BMI, and sex. Until such information becomes available, it would seem prudent not to reduce the dosages of the key drugs rifampin and isoniazid to below 450 mg and 300 mg daily, respectively, as these drugs are likely to be well tolerated at these dosages and lower dosages have not been adequately evaluated.

This pharmacokinetic study provides reassurance that the concentrations of the first-line antituberculosis drugs in ATT are not affected by ART comprising efavirenz, zidovudine, and lamivudine or by level of immune suppression. However, we found that males and patients who weigh the least are at risk of reduced drug exposure. Although our findings should be confirmed across populations representing the anthropometric variation of tuberculosis patients, they raise concern that current weight-based dosing strategies may contribute to suboptimal responses to ATT among patients with low BMIs, who are likely to include those with the most severe disease. Our findings suggest that revised weight band-based dosing recommendations for men and women would result in improved drug exposures.

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